URANYL PHOSPHATE MINERALS AS LONG TERM SINK IN URANIUM BIOREMEDIATION

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URANYL PHOSPHATE MINERALS AS LONG TERM SINK IN URANIUM BIOREMEDIATION

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CHAPTER 1

INTRODUCTION

Beginning with the Manhattan Project in 1942 and continuing through the end of the Cold War in early 1990's, the United States Department of Energy's nuclear fuel and weapon production has created an environmental and financial disaster (DOE, 1997). This era left the US with 3 million cubic meters of buried waste, which led to 40 million cubic meters of contaminated soil and 1.7 trillion gallons of contaminated ground water (NABIR, 2003), including 89% of waste by volume as a byproduct of uranium mining, milling, and refining (DOE, 1997). One site of contamination, the Oak Ridge Field Research Center (ORFRC), results from the dumping of acidic uranium nitrate in unlined waste disposal ponds (Brooks, 2001). Not only are uranium and nitrate concentrations high near the source, up to 252 μM and 645 mM respectively, they are also significant up to several hundred feet away as contaminants migrate, allowed for by subsurface bedrock, which, with soils, acts as a highly porous matrix (Jardine, 2006).

DOE has shifted its focus from nuclear production to remediation requiring cost effective techniques capable of handling this grand scale contamination. Traditional strategies primarily involve *ex situ* remediation strategies, such as pump-and-treat and excavation. Pump-and-treat extracts contaminated groundwater through monitoring wells for surface treatment. The dependence of this method on the advection of large volumes of water does little to ensure the containment of contaminants because processes, such as surface adsorption and precipitation, may prevent extraction. For soil remediation, excavation, in which contaminated soils are removed for treatment and disposal, is a time-consuming and costly method. Excavation, however, lacks efficiency, as redisposal

only moves contamination instead of removing it (Dawson and Gilman, 2001). Thus, a cost-effective and efficient approach to uranium remediation requires an *in situ* strategy (NABIR, 2003).

In situ remediation strategies include natural attenuations, which are cost-effective but not efficient, chemical remediation, which requires injection of often expensive chemicals in the subsurface, and biostimulation, which attempts to activate bacterial communities by pumping substrates in the subsurface (Jardine, 2006). In bioremediation, the solubility of metals, such as uranium, is decreased enzymatically by subsurface bacteria through anaerobic respiration or detoxification mechanisms (Lovley et al., 1991). Besides biological processes, metal solubility is also influenced by geochemical cycles. Thus, in situ biostimulation techniques must be assessed based on microbial processes coupled with hydrological and geochemical cycles (Jardine, 2006).

The most heavily researched *in situ* bioremediation technique is the bioreduction of water soluble uranium, U(VI), to insoluble uranium, U(IV) (North et al., 2004; Senko et al., 2002; Wade Jr. and DiChristina, 2000). Unfortunately, U(IV), as uraninite, is readily oxidized to the mobile uranyl ion, UO_2^{2+}, in oxic environments (Murphy and Shock, 1999). Furthermore, site geochemical conditions at ORFRC characterized by high nitrate concentrations of nitrate and low pH are unfavorable to uranium bioreduction (Beazley, 2007).

The biomineralization, or bacteria-mediated mineral precipitation, of water soluble U(VI) species is presented as a remediation alternative. Uranium in aerobic conditions, present as the uranyl ion, UO_2^{2+} (U(VI)), forms a stable bond with phosphate

reflective of the affinity of the uranyl ion for oxygen containing ligands and precipitates relatively insoluble uranium phosphate minerals (Wellman, 2005). Thus, immobilization of uranium is achieved through the formation of these minerals.

This study investigates the stability of these uranyl phosphate minerals to asses their potential as a long terms sink for uranium in the environment. This investigation includes the effect of cations and anions typically present in subsurface environments on uranyl phosphate mineral stability. Furthermore, the biomineralization of these minerals was examined in the presence of carbonates, which form highly soluble complexes with uranium(VI). The first part of this dissertation involves a literature review, while the second part presents the methods used in this study. The third section regroups the main results and discussion of these findings, including their implications for bioremediation studies. The last section synthesizes the results of this study and provides a list of questions to be addressed in future research.

CHAPTER 2

LITERATURE REVIEW

Geochemistry of subsurface

The fate of metal contaminants, especially as it relates to the redox state of metals, in the subsurface is governed by biological, geochemical, and hydrological processes. The subsurface consists of the vadose zone, which is the zone of aeration above the groundwater table, and the saturated zone, which is the zone below the water table, often associated with reducing conditions. Aerobic and anaerobic respiration by local microbial communities controls the geochemical conditions of these zones and ultimately influences contaminant speciation. In aerobic respiration, microbes utilize oxygen as the terminal electron acceptor (TEA), whereas anaerobic respiration involves alternative TEAs such as NO_3^-, iron oxides, manganese oxides, SO_4^{2-}, or even CO_2 or small organic acid substrates. TEAs are used based on availability and free energy gain. Oxygen provides the largest free energy gain, but its availability in the subsurface is a function of groundwater recharge through precipitation events (Stumm and Morgan, 1996). When oxygen is unavailable, NO_3^- becomes the TEA in another pertinent biological mechanism, denitrification, in which the reduction of NO_3^- to NO_2^-, NO, N_2O, and ultimately N_2 occurs. If NO_3^- concentrations are too low to drive anaerobic respiration processes, manganese and iron oxides are preferred as the TEA, then SO_4^{2-}, and eventually CO_2 or small organic acids for methanogenesis. Each respiration process is coupled to organic matter degradation and reoxidation, which generates dissolved

inorganic carbon, either under the form of CO_2 or HCO_3^-, depending on the natural pH and buffering capacity of the environment (Stumm and Morgan, 1996).

Contaminant transport is further influenced by geochemical processes, including adsorption and desorption, which refers to the molecular rearrangement of contaminants at the solid phase-aqueous interface (Stumm and Morgan, 1996), and reductive or non-reductive precipitation of minerals. Adsorption processes are controlled by the electrostatic surface charge of minerals, and thus the pH of the local environment. Above the pH of zero point charge (pH_{zpc}), mineral surfaces are on average negatively charged, and thus adsorb to positively charged species preferentially, while below the pH_{zpc}, mineral surfaces are positively charged and scavenge negative charges more favorable. Thus, adsorption of U(VI) to goethite, for example, is favorable between pH 5 and 7 when uranyl complexes are positively charged and goethite has a neutral surface charge (Langmuir, 1997). The association of uranium with goethite is observed at ORFRC as a transport controlling process (Stubbs, et. al., 2006).

General U Chemistry

Uranium exists most commonly as U(IV) and U(VI). U(IV) is found in reducing environments as uraninite, $UO_{2(s)}$, an insoluble species (Finch and Murakami, 1999). The electronic configuration of U(IV) is [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^6$ $5f^2$. Uraninite oxidizes to U(VI) as the uranyl ion, UO_2^{2+}, a soluble species (Rabinowitch and Belford, 1964). The electronic configuration of U(VI) is [Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^6$ $5f^0$. UO_2^{2+}, with the linear structure $[O=U=O]^{2+}$, maintains the axial oxygen bond configuration even in complexation reactions, during which equatorial ligands may be added (Cotton et al., 1999). The strength of these bonds is due to the O p orbital interaction with empty U

d and f orbitals (Burns, 1999). UO_2^{2+}, a hard acid, reacts with hard anions, such as oxygen containing ligands, and thus preferentially forms complexes with functional groups such as carboxylates and carbonyls (Suzuki and Banfield, 1999). Greater stability is exhibited by polydentate ligands than monodentate ligands (Stumm and Morgan, 1996).

One such ligand, carbonate, CO_3^{2-}, forms a stable complex with U that dominates U speciation at circumneutral pH (Finch and Murakami, 1999). The influence of carbonate on U speciation, as it displaces other ligands, is limited to neutral to basic environments, because it is protonated below pH 6.35, and thus not available for complexation. Calcium, a common cation in the subsurface, forms complexes with U(VI) and CO_3^{2-} to form species that increase the rate of mineral dissolution and decrease bioreduction (Liu et al., 2007). Not only do uranyl carbonate species promote uranyl mineral dissolution, they also prevent U adsorption to mineral surfaces (Langmuir, 1978). Abiotic *in situ* uranium remediation techniques, including adsorption to mineral surfaces, mineral coprecipitation, and complexation with organic compounds, have been examined (Beazley, 2009).

Bioremediation of Uranium

Biotic *in situ* strategies have focused heavily on bioreduction, the enzymatic reduction of soluble U(VI) to insoluble U(IV) (North et al., 2004), by dissimilatory metal reducing and sulfate reducing bacteria, including *Shewanella*, *Geobacter*, *Desulfovibrio*, and a few newly discovered microorganisms (Ginder-Vogel, et. al., 2006). The limitations of bioreduction include circumneutral pH, occurring optimally above

pH 7 (Wu, et. al., 2006) and requires anaerobic conditions, as the product of reduction, uraninite, is readily oxidized in the presence of oxygen, nitrite (the product of dentrification in nitrate rich conditions), Mn oxides, and Fe(III) (hydr)oxides (Ginder-Vogel, e. al., 2006).

Interestingly, the mechanism of bioreduction remains poorly defined. Fe(III) reducing bacteria are able to reduce U(VI) while also conserving energy for growth, whereas sulfate reducers that can also reduce U(VI) are not able to conserve energy from this process (Llyod, 2003). Reduction is demonstrated to occur with electron transfer between uranium and a *c* type cytochrome, and either occurs through a two electron transfer, reducing U(VI) to U(IV), or through a one electron transfer in which a U(V) intermediate is formed. The final product of reduction is the insoluble U(IV) species, uraninite, which precipitates (Lloyd and Renshaw,2005). In addition to the pH conditions and the presence of oxidants of uraninite, the bioreduction of U(VI) is limited at ORFRC due to the high nitrate conditions. Microbes exploit terminal electron acceptors in order of free energy gain (DiChristina, et. al., 2005) and nitrate offers a higher free energy gain for bacteria and is, thus, preferentially used as the terminal electron acceptor even in the presence of U(VI) (Wall and Krumholz, 2006).

Biomineralization

Because bioreduction is limited of by the geochemistry of the subsurface, a redox independent uranium remediation strategy must be developed. The biomineralization of water soluble U(VI) species is presented as a remediation alternative. Uranium, present as UO_2^{2+} in aerobic conditions, forms a stable bond with phosphate reflective of the affinity of the uranyl ion for oxygen containing ligands. Uranium phosphate minerals are

relatively insoluble uranyl minerals (Wellman, et. al., 2005). However, free orthophosphate exists in low concentrations in groundwater, and addition of orthophosphates in aquifers is not a feasible strategy, as adsorption and precipitation of calcium phosphate minerals are so rapid that uranium phosphate precipitation occurs only near the source of phosphate (Wellman, et. al., 2006). In U(VI) contaminated subsurfaces, the microbially mediated hydrolysis of organophosphates has been demonstrated to provide an alternative source of phosphate for precipitation of insoluble uranyl-phosphate phases (Beazley, et. al., 2007). This biological mechanism prevents U toxicity by taking uranium out of solution, thereby providing cellular protection and a competitive advantage over other microorganisms (Martinez, et. al., 2007). Inorganic phosphate may be liberated microbially through phosphatase activity, both in acidic (Rossolini et al., 1998) and alkaline conditions (Nilgiriwala, et. al., 2008). Microorganisms expressing phosphatase activity may also express metal resistance (Macaskie et al., 1992). *Rahnella*, a metal resistant strain isolated from ORFRC, is able to precipitate 95% of U(VI) in aerobic (Beazley, et. al., 2007) and anaerobic conditions (Beazley, 2009). Thus, a metal resistant bacterium with phosphatase expression in both aerobic and anaerobic environments may represent an advantageous bioremediation strategy, even though it requires source of exogenous organic phosphorous (Beazley, et. al., 2007).

Objectives

While the role of different organophosphate species and bacterial strains in the biomineralization of uranium continues to be studied, the stability of these uranyl phosphate minerals must also be examined to asses their role as long term sinks for

uranium (Smeaton, et. al., 2006). This study investigates the stability of these uranyl phosphate minerals to assess their potential as a long terms sink for uranium in the environment. This investigation includes the effect of cations and anions typically present in subsurface environments on uranyl phosphate mineral stability. Furthermore, the biomineralization of these minerals was examined in the presence of carbonates, which form highly soluble complexes with uranium(VI), to determine whether carbonates are detrimental to the immobilization of uranium under the form of phosphate minerals.

CHAPTER 3

METHODS

Experiments were performed to asses the stability of the uranyl phosphate mineral in the presence of environmentally relevant cations and anions, as well as in the presence of carbonates. Site conditions were simulated by conducting experiments in simulated groundwater (SGW) containing 2 μM $FeSO_4$, 5 μM $MnCl_2$, 8 μM Na_2MoO_4, 0.8 mM $MgSO_4$, 7.5 mM $NaNO_3$, 0.4 mM KCl, 7.5 mMKNO_3, and 0.2mM $Ca(NO_3)_2$.

The effect of environmentally significant ions on the solubility of the uranium phosphate mineral was examined. Chernikovite ($H(UO_2)(PO_4)\cdot 4H_2O$) was precipitated abiotically in SGW, 200 μM uranyl acetate (Spectrum), and 1 mM monobasic sodium phosphate, NaH_2PO_4 (Sigma). Chernikovite suspensions were equilibrated on a rotary wheel for 24 hours, then centrifuged at 3300 rpm for 10 minutes to separate the precipitate from the supernatant. The supernatant was removed, the mineral precipitate was rinsed twice with Nanopure water (Barnstead). The mineral was then resuspended in SGW amended with NaCl, K_2SO_4, or $CaCl_2$ at varying concentrations within a range of concentrations consistent with the geochemical conditions at the ORFRC site. Stability was examined at pH 8, using a buffered solution containing 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and NaOH, pH 7 also using 50 mM HEPES

adjusted with NaOH, pH 5.5 using 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) and NaOH, and pH 4.5, an unbuffered solution. The suspensions were allowed to equilibrate for 10 days on a rotary wheel, after which the supernatant was separated and analyzed for dissolved U(VI) and phosphate (see below).

The biotic precipitation of uranyl phosphate mineral in the presence of carbonates was also examined. The metal-resistant subsurface bacterial strain used in this study, *Rahnella* sp. Y9602, exhibited phosphatase-positive phenotypes (Martinez et al., 2007) Cells were maintained in nutrient broth (NB) agar (3 g beef extract, 5 g peptone, 15 g agar per liter). Cells were initially cultivated overnight at 30^{o}C in NB agar (pH 6.8) from frozen stocks (-80^{o}C), then were grown for 16-18 hr in NB broth (pH 5.5) and subsequently diluted 1/25 into fresh NB (pH5.5) and regrown to mid-log phase. Cells were harvested, for a final concentration of 10^7 cells, by centrifugation (10 min), washed twice with isotonic saline (8.5 g L^{-1} NaCl), and resuspended in SGW (pH 5.5 and 7) to a final concentration of 10^7 cells, amended with 10 mM glycerol-2-phosphate (G2P) (Sigma-Aldrich) as the sole phosphate source, X mM Glycerol as carbon source, and either with or without $NaHCO_3$. Each sample was incubated aerobically in duplicates by constantly mixing the suspensions in open Erlenmeyer flasks in a shaker at 25^{o}C. Biotic samples required the use of sterile techniques to maintain pure cultures. Abiotic controls served as indicators that experimental conditions remained asceptic, as well as a control for U transformations without microbial influence. Preliminary abiotic experiments were conducted to ensure the absence of U-G2P mineral formation in the conditions of the incubations. These experiments were performed at pH 5.5 and pH 7, using MES and

HEPES respectively, with 200 uM U as initial concentration, and varying concentrations between 0 mM and 10 mM G2P.

Dissolved U(VI), concentrations were measured using an Agilent 7500a Series system for inductively coupled plasma-mass spectrometry (ICP-MS). Samples were centrifuged, and the supernatant was filtered (0.2 μm pore size, AcetatePlus; GE Water and Process Technologies) and acidified with 2% nitric acid (trace metal grade, Fisher) diluted in Nanopure water (Barnstead). Holmium and bismuth (SPEX certiPep) were used as internal standards and River Water Certified Reference Material for Trace Metals (SLRS-4, National Research Council Canada, Ottawa, Canada) for quality controls (Figure 1). Calibration curves were rerun every 20 samples to account for instrument drift.

Dissolved phosphate was measured by spectroscopy (Murphy, 1962). The molybdate reagent was freshly prepared using 40 uM ascorbic acid, 280 uM potassium antimonyl tartrate, 933 uM sulfuric acid, and 3.1 uM ammonium molybdate. An aliquot of the reagent, 50 uL, was added to 500 uL of the filtered supernatant. The color was allowed to develop for twenty minutes followed by spectrophotometric detection at 885 nm (Milton Roy Spectronic 501 spectrophotometer).

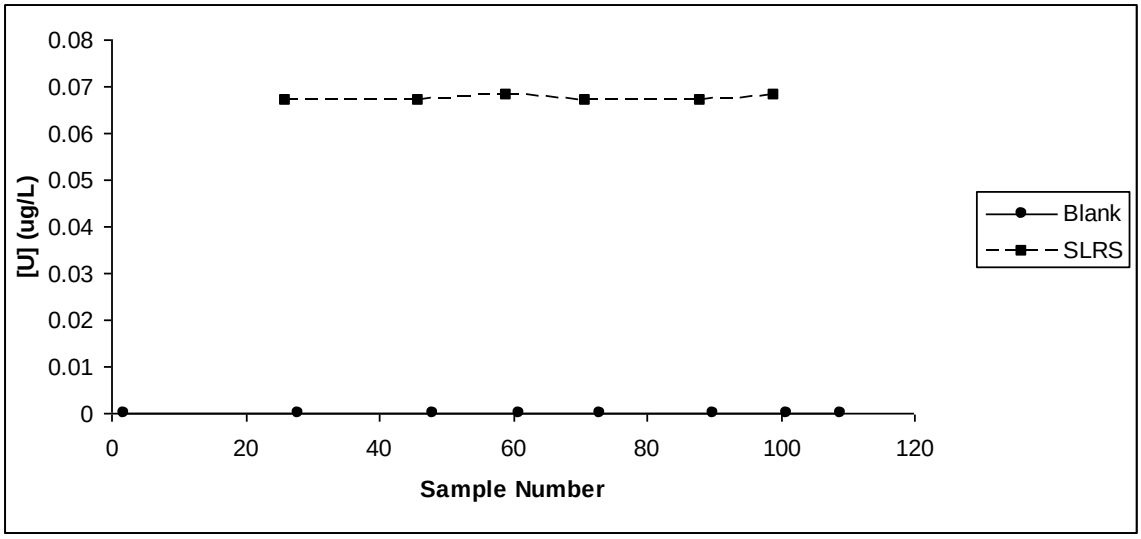


Figure 1. ICP-MS Quality Controls: Blank and SLRS Concentrations as a Function of Sample Number. SLRS concentrations are highly reproducible but about 30% higher than the certified concentration, probably because it is close to the detection limit obtained with the calibrations used in this study (first standard was 50 ug/L in Figure 2).

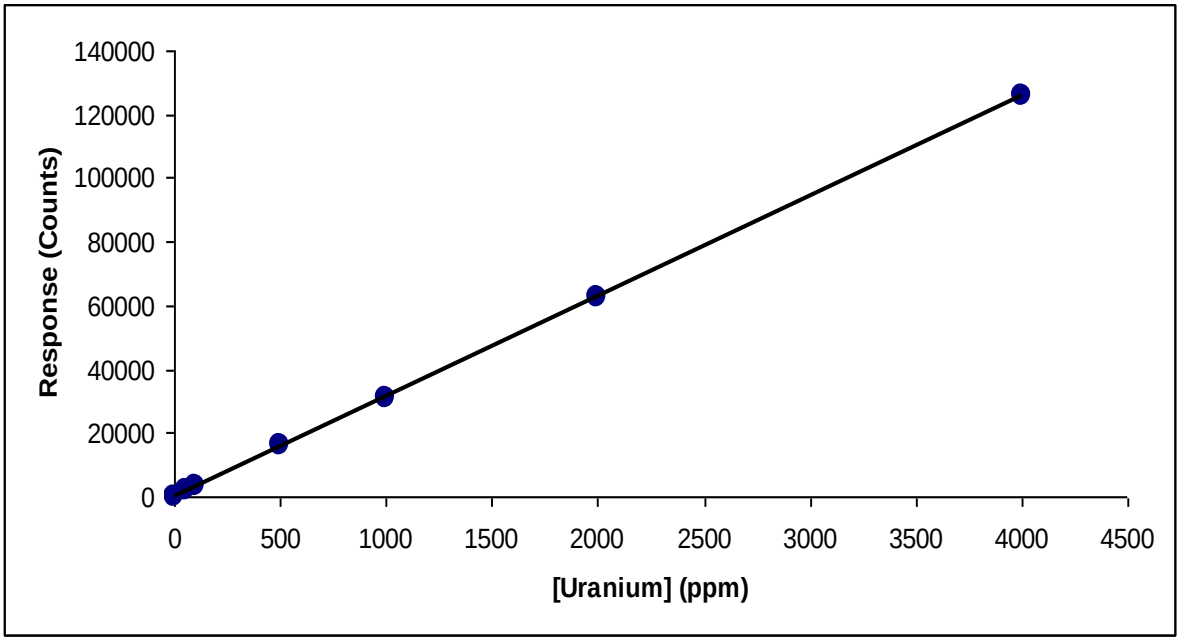


Figure 2. Representative Calibration Curve from Uranium Analyses by ICP-MS. Relationship is given by y= 31.459± 0.05.

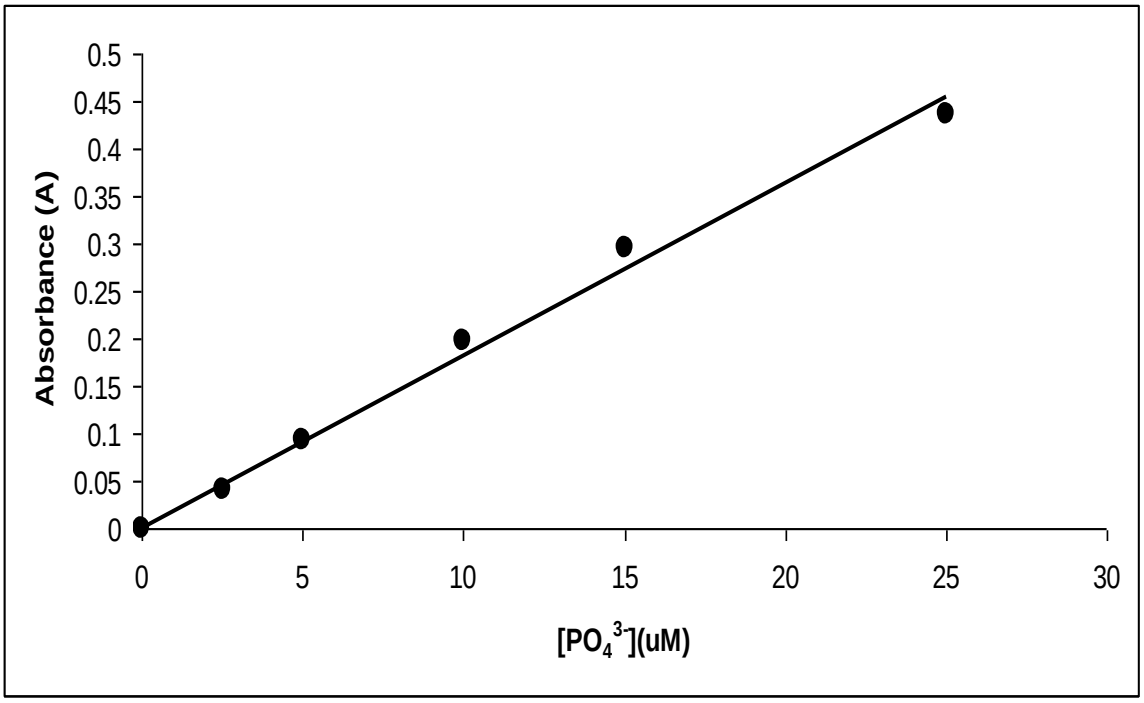


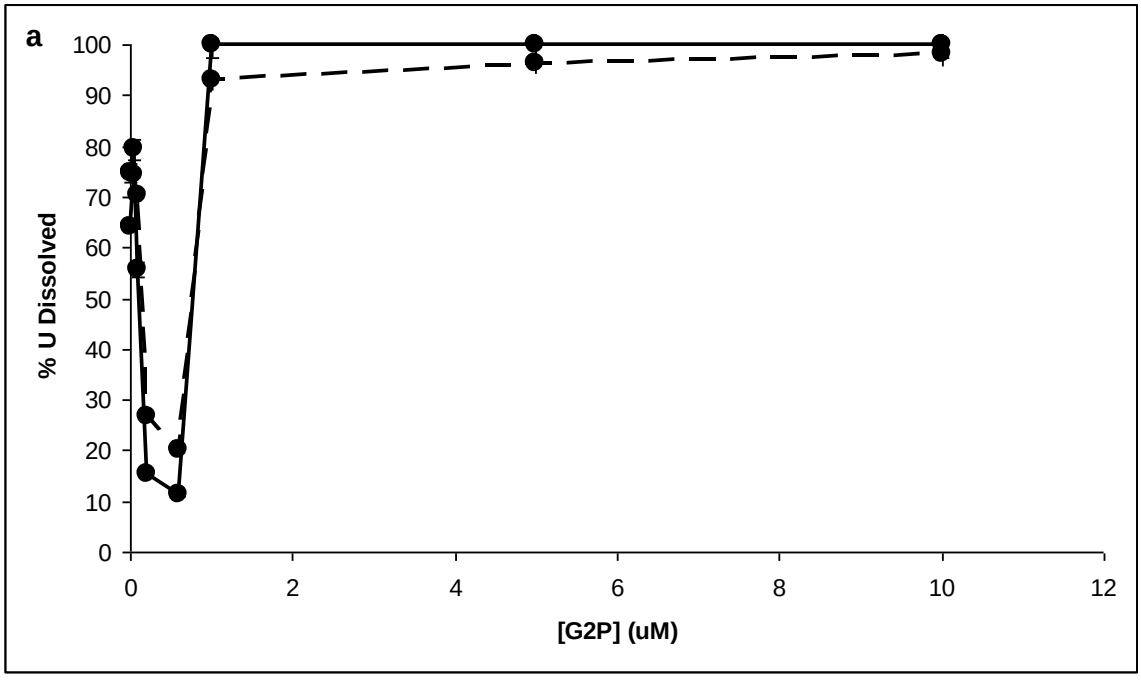
Figure 3. Representative Calibration Curve from Phosphate Analyses by Spectrophotometry. Relationship is given by y= 0.0182 ± 0.004.

CHAPTER 4

RESULTS AND DISCUSSION

The solubility of U(VI) in the presence of exogenous organophosphate was examined at pH 5.5 and pH 7 with and without the presence of carbonates to ensure that U(VI) used in the experiments with microorganisms did not precipitate abiotically with the organophosphate (Figure 4). At pH 5.5, carbonates have little effect on the solubility of uranium. Below a pH of 6.35, H_2CO_3 is the dominant carbonate species in solution (Stumm and Morgan, 1996) and should, thus, not be available for complexation. In addition, most of the dissolved inorganic carbon probably degasses into the atmosphere at such a pH. At G2P concentrations ranging between 0.2 and 0.6 mM, U(VI) is removed from solution, possibly under the form of mono- to tridentate complexes of U(VI) with

G2P. At greater G2P concentrations, however, U(VI) remains completely soluble in SGW, likely because the accumulation of negatively charged G2P onto U(VI) increases steric hindrance and repulsive forces that destabilize the U(VI) precipitate. At pH 7, the solubility of U(VI) is limited even without G2P, as uranyl hydroxide minerals precipitate at this pH in the absence of orthophosphate (Langmuir, 1997). Simultaneously, the solubility of U(VI) is low at G2P concentrations lower than 1mM (Figure 4b), though in a less dramatic fashion than at pH 5.5 (Figure 4a). Interestingly, the solubility of U(VI) is enhanced in the presence of carbonates at pH 7. At this pH, the dominant carbonate species is HCO_3^-, which forms soluble complexes with U(VI) (Finch and Murakami, 1999). This increase in U(VI) solubility at pH 7 suggests uranyl hydroxide minerals, the dominant mineral at neutral pH in the absence of orthophosphates, are prevented to form by carbonates. Subsequently, G2P concentrations of 10 mM were used in all biotic experiments to ensure U precipitation represented mineral formation with orthophosphate as a result of the biotic hydrolysis of G2P only.



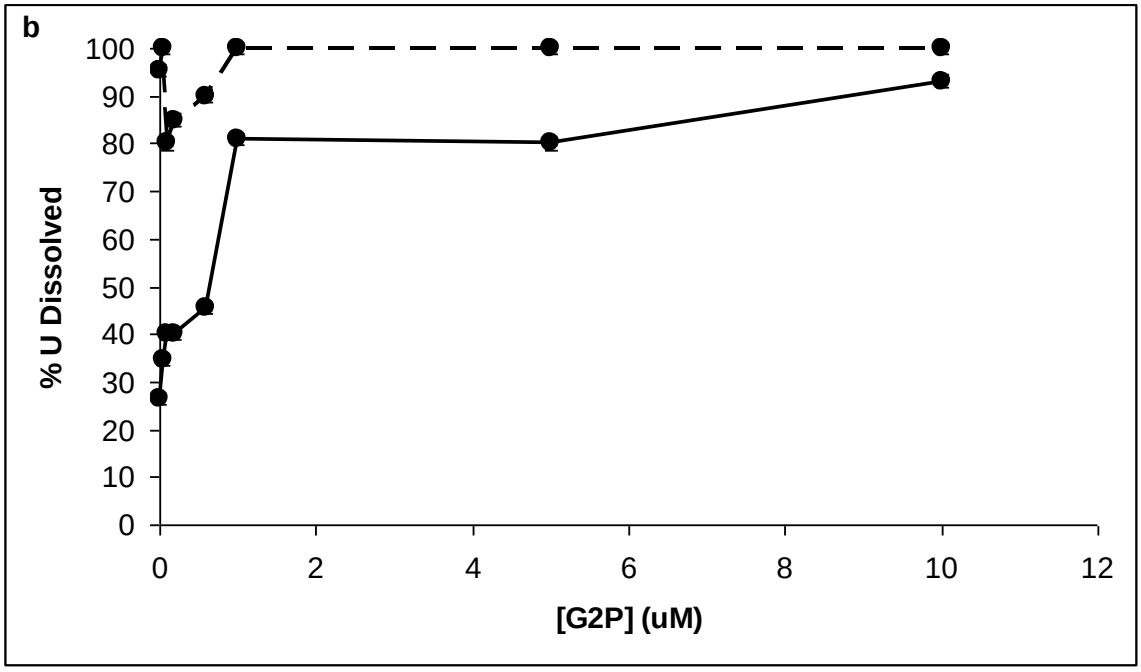


Figure 4. Solubility of 200 uM U(VI) as a function of G2P Concentration with10 mM (dashed line) and without (solid line) $NaHCO_3$ at pH 5.5 (a) and at pH 7 (b). Error bars represent variation in duplicates, and instrumental and analytical error.

Aerobic incubations with *Rahnella* sp. were also performed in the presence of varying concentrations of uranium initially (Figure 5) to determine whether U(VI) may be toxic to the microorganism. Phosphate released through the hydrolysis of G2P was analyzed as a proxy for respiration. Incubations were analyzed after 36 hours. Flasks with U(VI) concentrations less than 10 uM appeared turbid, indicating that microorganisms were able to grow, while the 100 uM, 1 mM U(VI), and above incubations remained clear and colorless, suggesting the addition of such high concentrations of U(VI) was detrimental to the organisms. Dissolved uranium was not measured, but would indicate whether or not phosphate release is limited by uranyl phosphate mineral precipitation rather than inhibited as a result of microbe intoxication. Instead, the saturation index of chernikovite in the incubation conditions was calculated to determine if the decline in phosphate release was due

to precipitation or intoxication of the cells by uranium in solution. Chernikovite precipitation may be described as

$$((UO_2)_2(HPO_4)_2)\cdot 4H_2O(s) \rightleftharpoons 2UO_2^{2+} + 2PO_4^{3-} + 2H^+ + 4\,H_2O \qquad \text{I}$$

with a $\log K_{sp}$ value of -45.9 for the reaction (Grenthe, et. al., 1992). Thus, assuming P_{Total} to be 1.3 mM based on the phosphate released in the control after 36 hours, $[PO_4^{3-}]$ may be calculated at pH 7 using mass balance, the acid dissociation constants for each of the phosphate species, and assuming H_3PO_4 was negligible at pH 7. $[PO_4^{3-}]$ was determined to be 2.1 nM. Thus, the saturation index may be quantified according to the ratio

$$\Omega = \frac{Q}{K_{sp}} \qquad \text{II}$$

where Q represents the solubility constant of the reaction in experimental conditions, assuming the solutions to be close to ideal.

$$Q = [UO_2^{2+}]^2[PO_4^{3-}]^2[H^+]^2 \qquad \text{III}$$

A Ω value less than 1 indicates that the system is undersaturated with respect to chernikovite formation. Thus, the incubations with 100% release of dissolved phosphate were undersaturated with respect to chernikovite formation, while the incubations that produced much less phosphate compared to the control were determined to be supersaturated (Figure 5). Supersaturation suggests that the decrease of phosphate release may have been attributed to uranyl phosphate precipitation. Thus, the microorganism was likely able to sustain growth at U(VI) concentrations less or equal to 100 uM, but not above that concentration.

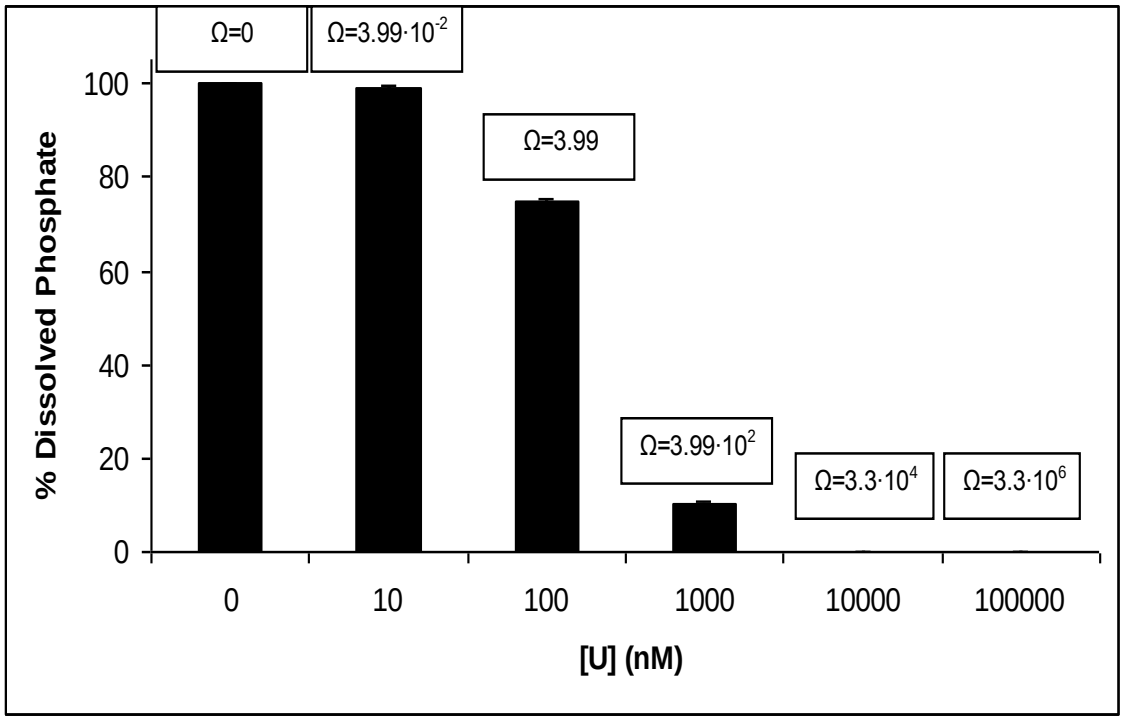


Figure 5. Percent orthophosphate released in solution after 36 hours during hydrolysis of 10 mM G2P by *Rahnella* sp. in the presence of different initial uranium concentrations. The fractions of phosphate released are normalized to phosphate released by the control (0 nM uranium). The saturation index (Ω) is provided above each incubation to infer eventual precipitation of chernikovite. Error bars represent variation in duplicate incubations, and instrumental and analytical error.

The effect of carbonates on the biomineralization of uranyl phosphate minerals by *Rahnella*, sp. was determined in a series of duplicate aerobic incubations at pH 7. Uranium was added to selected incubations at 36 hours. Incubations conducted with 200 uM uranium amended initially did not produce phosphate (not shown) and did not consume U(VI) (Figure 6), compared to incubations amended with U(VI) after 36 hours (Figures 6 and 7). These results confirm that U(VI) prevents initial cell growth, as predicted by the toxicity experiments (Figure 5). Phosphate analyses reveal lower dissolved phosphate

produced in incubations containing carbonates, even in the absence of U(VI) (Figure 7) suggesting that carbonates may interfere with the thermodynamic driving force of the reaction and slow down respiration (Jin and Bethke, 2003). Furthermore, phosphate production reaches a steady-state after addition of uranium in the incubations without carbonates, while production of phosphate continues when carbonates are present, albeit at lower rates than without U(VI) (Figure 7). Phosphate is consumed during precipitation of uranium phosphates after addition of U(VI) at 36 hours, regardless of the presence of absence of carbonates (Figure 6), suggesting that the complexation of U(VI) by carbonates decreased the toxicity of uranium. Metal toxicity is often described by the 'free' ion activity model, which assumes that free hydrated metals only are taken up by cells (Tessier, et. al., 1994). Thus, the presence of carbonates may lower the concentration of 'free' hydrated or monohydroxylated uranyl ion in solution, lower uranium toxicity, and could explain the continuous increased production of phosphate compared to incubations without carbonates, despite the fact that respiration is generally less efficient in the presence of carbonates.

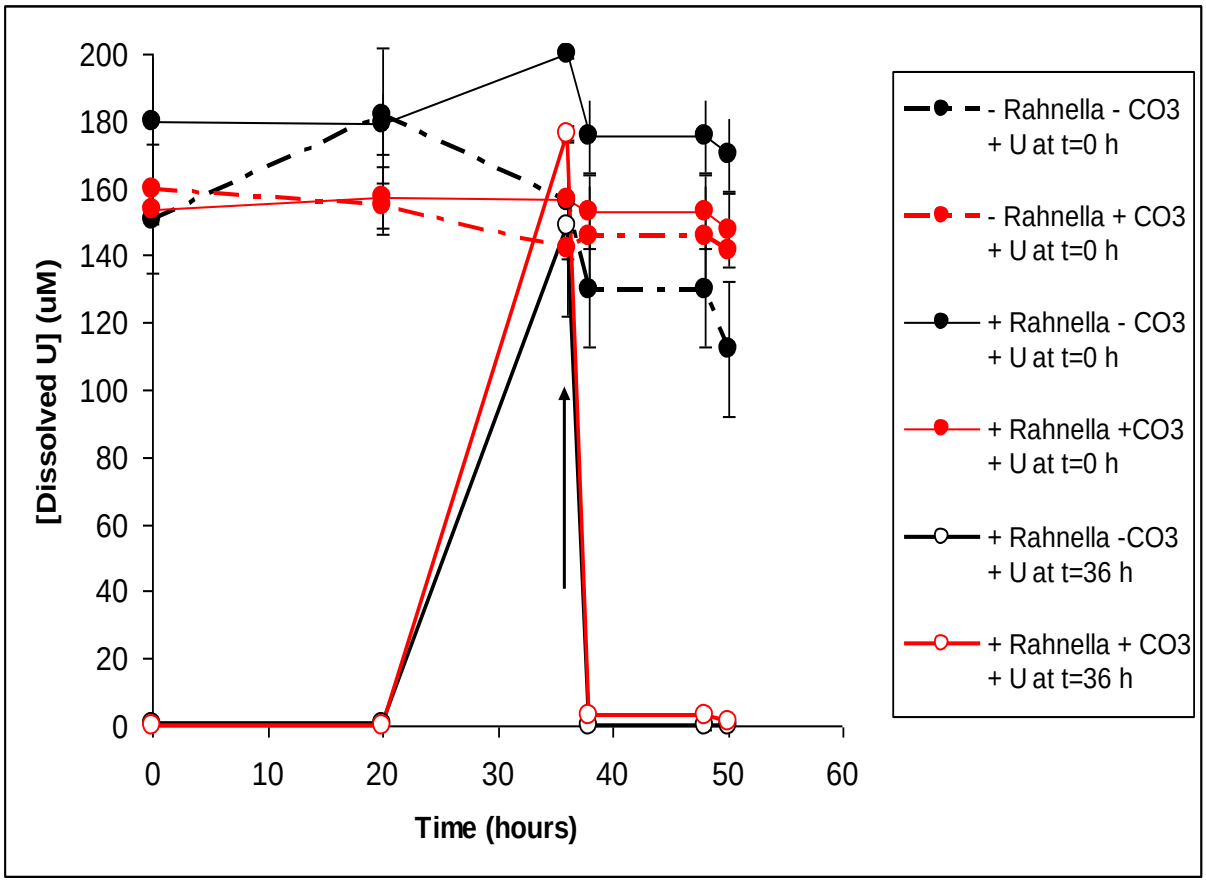


Figure 6. Dissolved uranium in incubations containing 10 mM G2P, 200 uM U(VI) either added initially (closed symbols) or at 36 hours (open symbols) in the presence (red) or not (black) of 10 mM carbonates, and inoculated (solid line) or not (dashed line) with *Rahnella*, sp. Arrow indicates U(VI) addition. Error bars represent variation in duplicates, and instrumental and analytical error.

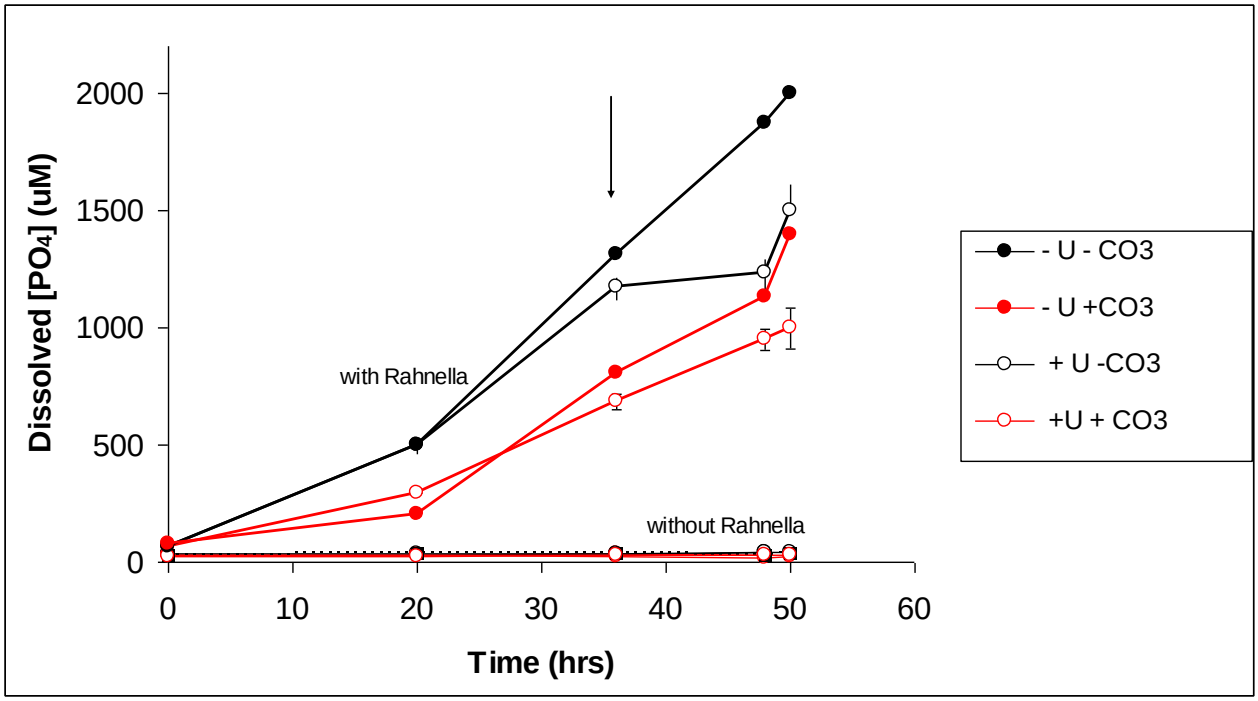


Figure 7. Total dissolved phosphate released during hydrolysis of 10 mM G2P by *Rahnella*, sp. in media amended (red) or not (black) with 10 mM carbonates, and in the presence (open) or not (closed) of 200 uM U(VI). Abiotic controls without *Rahnella*, sp. are provided for reference. Arrow indicates U(VI) addition. Error bars represent variation in duplicates, and instrumental and analytical error.

The stability of the uranyl phosphate mineral was also examined when exposed to anions and especially cations, as the protons of chernikovite can easily be substituted by monovalent and divalent cations to form autunites and meta-autunites (Lacock, et. al., 2004). In all of these experiments, chernikovite was rinsed before exposed to simulated ground water, such that the release of uranium at no addition of anion or cation is likely due to the equilibration of the mineral with the undersaturated solution. Sulfate slightly increased the stability of the uranium phosphate mineral at all pH (Figure 8), as expected as sulfate does not form strong complexes with uranyl ions (Langmuir, 1997) and is

known to inhibit mineral dissolution (Stumm, 1997). At low sodium and calcium additions, the solubility of uranium phosphate minerals is enhanced at low pH, likely due to destabilization in the presence of excess protons. Sodium, however, appears to have an overall stabilizing effect on the mineral over a range of concentrations and pH (Figure 9), while calcium stabilizes the uranium phosphate mineral below a concentration of 150 mM independently of the pH and increases dissolution above 150 mM (Figure 10). In turn, potassium appears to have little effect on the mineral at any pH (Figure 11). The mechanism by which calcium or sodium replace the hydrogen in the uranyl phosphate mineral is most likely ion exchange (Vochten et. al., 1992; Lopock, et. al., 2004), as Na^{+} and especially Ca^{2+}, have stronger affinities for minerals than protons. The addition of excess Ca^{2+}, however, must result in an excess of positive charges on the surface layer which favors repulsion, and thus, dissolution of the meta-autunite mineral, while the addition of excess Na^{+} should not affect the dissolution of the Na-autunite, as alkaline elements do not adsorb significantly on mineral surfaces. K^{+} has a larger radius than Na^{+} (Cotton et al., 1999) and may be more difficult to substitute for protons than sodium, thus explaining the little effect of potassium on the solubility of chernikovite. Alternately, potassium may substitute for the protons, but the stability of the potassium autunite may be decreased due to combined effect of the increase in acidity of the solution upon ion exchange and the higher solubility of the potassium autunite mineral. Furthermore, the apparent increased in stability of the uranium phosphate mineral at pH 5.5 compared to pH 4.5 must result from the relationship between dissolution rate and surface charge. The minimum rate of dissolution coincides with the pH_{zpc} of autunite minerals, which is around 5 and 6 (Wellman et al., 2007). Dissolution rate is related to surface charge;

dissolution rate increases with increasing negative or positive charge due to H^+ or OH^- in solution, respectively (Wellman, et. al., 2007). This trend indicates biomineralization as a favorable mechanism as the phosphatase activity of *Rahnella* strains reach optimal activity at pH of 5.0-5.5 (Martinez, et. al., 2007).

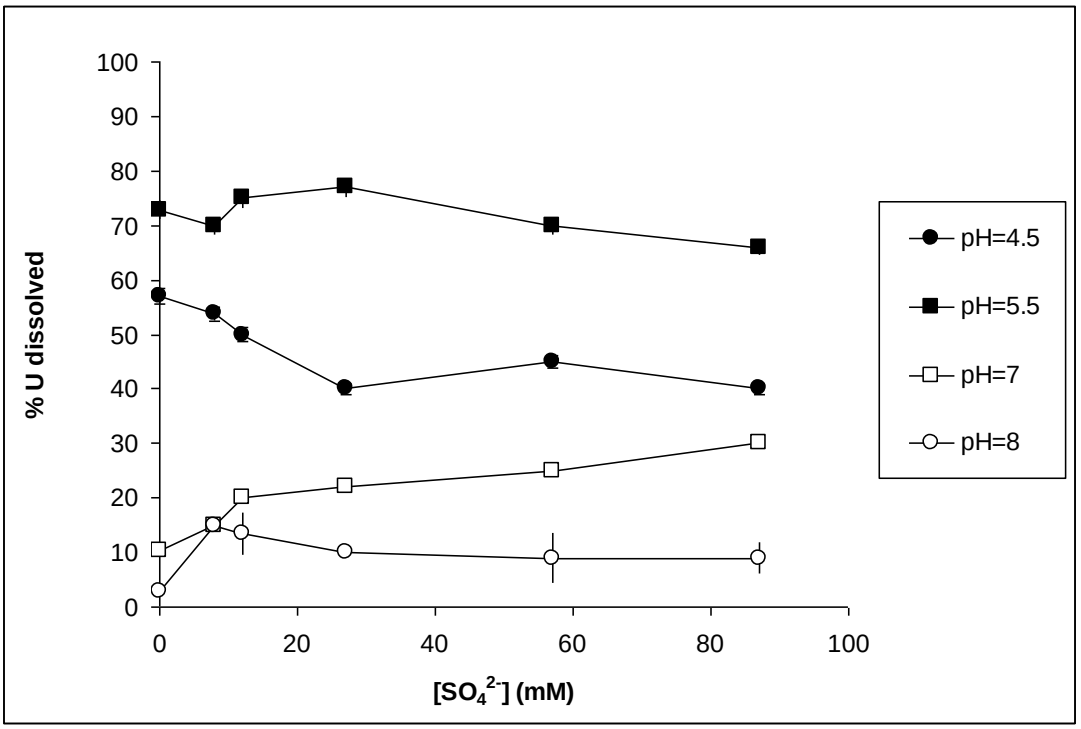


Figure 8. The dissolution of abiotic chernikovite by sulfate represented as the percentage of dissolved uranium produced at equilibrium (10 days) as a function of the concentration of dissolved K_2SO_4 at pH 4.5, 5.5, 7.0 and 8.0 in simulated groundwater.

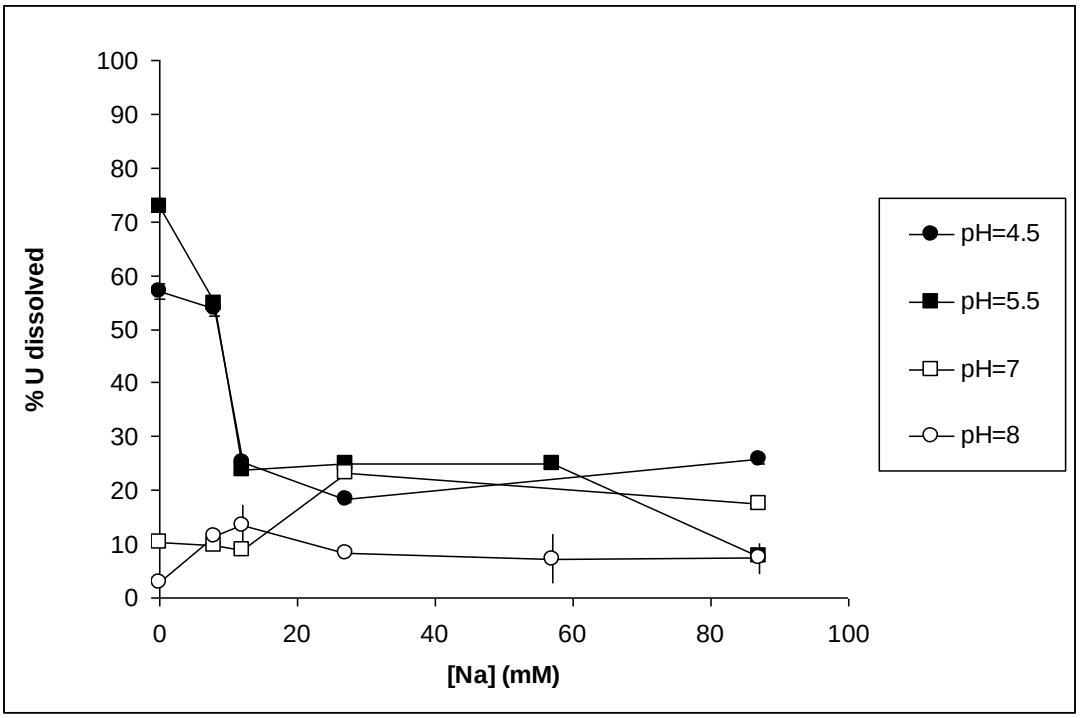


Figure 9. The dissolution of abiotic chernikovite by sodium represented as the percentage of dissolved uranium produced at equilibrium (10 days) as a function of the concentration of dissolved NaCl at pH 4.5, 5.5, 7.0 and 8.0 in simulated groundwater.

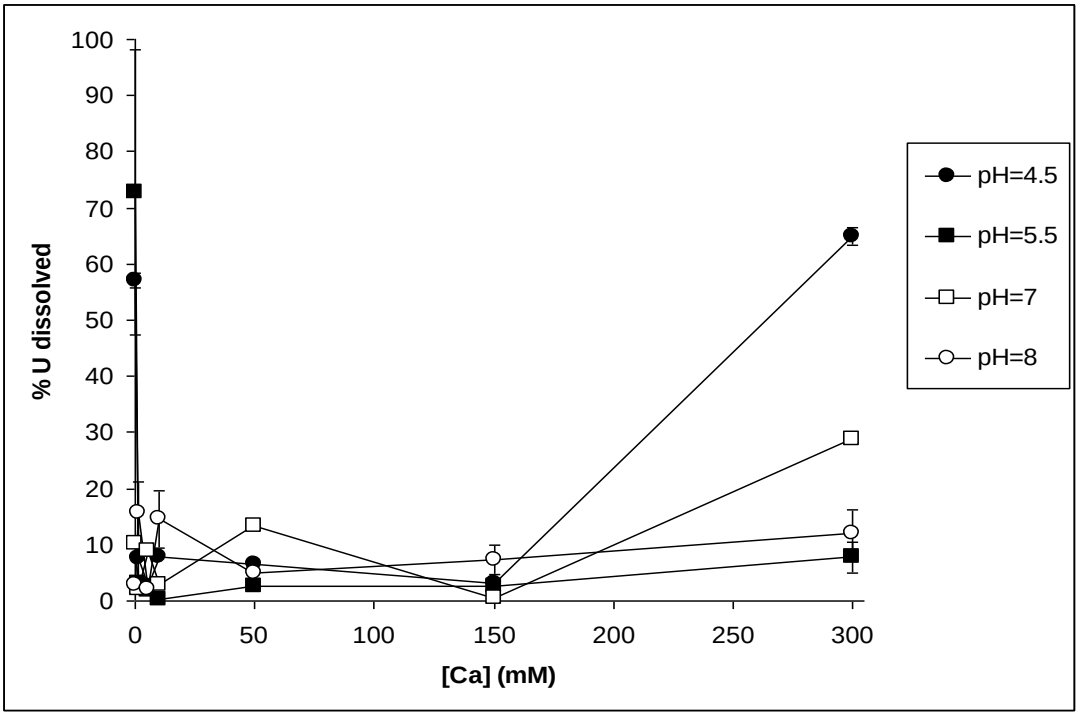


Figure 10. The dissolution of abiotic chernikovite by calcium represented as the percentage of dissolved uranium produced at equilibrium (10 days) as a function of the concentration of dissolved $CaCl_2$ at pH 4.5, 5.5, 7.0 and 8.0 in simulated groundwater.

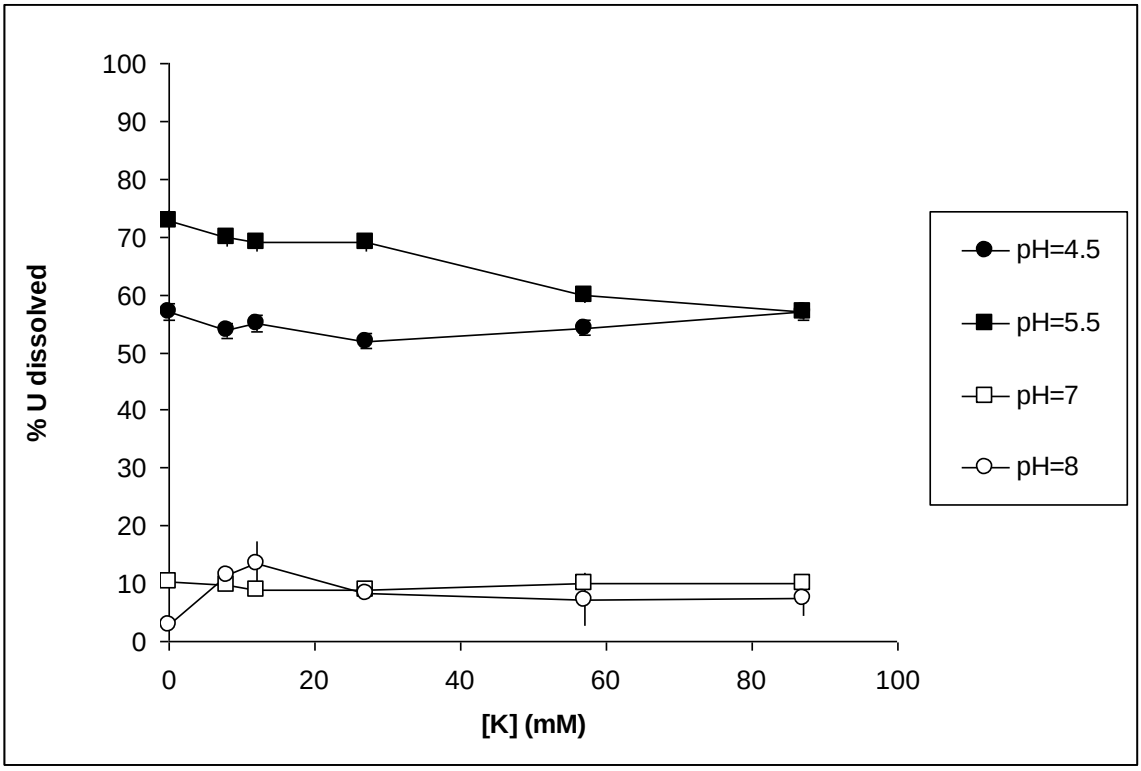


Figure 11. The dissolution of abiotic chernikovite by potassium represented as the percentage of dissolved uranium produced at equilibrium at equilibrium (10 days) as a function of the concentration of dissolved KCl at pH 4.5, 5.5, 7.0 and 8.0 in simulated groundwater.

CHAPTER 5

CONCLUSION

The gigantic uranium contaminations in the Department of Energy sites across the country cannot be remediated by conventional ex situ techniques, and such efforts have to rely on in situ strategies that have to be optimized. Bioremediation techniques, which aim at activating in situ microbial processes to immobilize uranium have been promising, but the bioreduction of uranium requires circumneutral pH conditions, may be outcompeted by better terminal electron acceptors, and is susceptible to reoxidation by a variety of oxidants. Thus, the geochemical conditions of the sites have to be considered when designing a bioremediation strategy. The geochemical conditions at ORFRC require a redox independent, *in situ* remediation strategy for uranium immobilization as the pH is too low and nitrate concentrations too high to facilitate the bioreduction of uranium. Studies with pure cultures and contaminated soils, have shown that the biomineralization of uranium, through its immobilization in the form of insoluble uranium(VI) phosphate minerals, offers an efficient alternative, providing microorganisms carrying phosphatases may be stimulated in situ. This study explored the stability of the uranyl phosphate mineral in the presence of common anions and cations in natural waters to determine whether this strategy would ultimately stabilize uranium in the solid phase. The precipitation of biogenic uranyl phosphate minerals through the hydrolysis of G2P by *Rahnella*, sp. proved to be facilitated even in the presence of carbonates, which typically form strong complexes with U(VI) and, in high concentrations, destabilize uranium phosphate minerals. More importantly, carbonates apparently decrease the toxicity of U(VI) at circumneutral pH, likely because carbonate complexes are not bioavailable, thus

allowing the cells to hydrolyze more organophosphate for uranium biomineralization. Abiotic uranyl phosphate minerals were exposed to environmentally relevant cations to determine their effect on the stability of chernikovite, the mineral identified to form in the presence of orthophosphates. Sodium and calcium were found to stabilize the mineral, though mineral dissolution is promoted at high calcium concentrations due to excess positive charge on the mineral surface. In turn, potassium had no apparent effect on the dissolution of chernikovite, likely because this monovalent cation is too large and not strong enough to displace the protons of chernikovite. Overall these findings demonstrate that the biomineralization of uranium phosphate minerals may represent an alternative bioremediation strategy to the bioreduction of uranium. Additional work could include further characterizing the mineral formed in SGW conditions of varying cation concentrations, investigating the toxicity of uranium on *Rahnella* sp. in the presence of carbonates, and examining the biomineralization of uranium phosphate minerals at low uranium concentrations to determine whether supersaturation is reached even at non-toxic uranium levels.

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